Multiplexed genetic engineering of human hematopoietic stem and progenitor cells using CRISPR/Cas9 and AAV6.

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Public Summary:
Precise and efficient manipulation of genes is crucial for understanding the molecular mechanisms that govern human hematopoiesis and for developing novel therapies for diseases of the blood and immune system. Current methods do not enable precise engineering of complex genotypes that can be easily tracked in a mixed population of cells. We describe a method to multiplex homologous recombination (HR) in human hematopoietic stem and progenitor cells and primary human T cells by combining rAAV6 donor delivery and the CRISPR/Cas9 system delivered as ribonucleoproteins (RNPs). In addition, the use of reporter genes allows FACS-purification and tracking of cells that have had multiple alleles or loci modified by HR. We believe this method will enable broad applications not only to the study of human hematopoietic gene function and networks, but also to perform sophisticated synthetic biology to develop innovative engineered stem cell-based therapeutics.

Scientific Abstract:
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